THE DIGESTION OF BEEF PROTEINS IN THE HUMAN STOMACH[1]

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It is at least 2500 years since man first exhibited curiosity about the mechanism of the process by which he digests his food. Since that time the stomach has been regarded as playing, if not the most important, at least a very important part in the process. It is a curious fact that, while as a result of considerable research since the early seventeenth century, we know a great deal about the part played by that portion of the digestive tract described as the intestines, we know little or nothing of the part performed by the stomach itself. It is true, of course, that an immense amount of information has been gained in the last quarter of a century about gastric secretions and gastric motility. Extensive investigations have also been made into the mechanism and the nature of the products of peptic digestion *in vitro,* but little or no knowledge has been gained of the extent and nature of gastric digestion of protein in the living stomach. Still less is known of disturbances which may occur in the gastric digestive process in disease, although in at least one disease it seems possible that disturbance of this process may play an important rôle. The most striking precursor and concomitant of pernicious anaemia is the condition described as achlorhydria. It has been known that achlorhydria is accompanied in many cases by achylia and it is a corollary therefore that in pernicious anaemia gastric digestion is incomplete or entirely absent. There have been no systematic studies of gastric digestion of protein hitherto made in this disease.

In view of the results obtained by Castle and others (1) in the treatment of pernicious anaemia with normal gastric digests and extracts of gastric and other tissues, which suggest that faulty gastric secretion and digestion may be at least a factor in the causation of the disease, it was deemed desirable to carry out an investigation of the extent and character of gastric protein hydrolysis in normal individuals and in a number of abnormal conditions, but especially in simple achlorhydrics and in patients with pernicious anaemia.

A number of preliminary experiments *in vitro* were essential to establish the experimental conditions. These are described below under the

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following headings: (1) the rate of hydrolysis; (2) the error introduced by the momentary acceleration of hydrolysis due to increased temperature when, in determining the amount of hydrolysis of beef protein during a given time, boiling was employed to destroy the enzyme; (3) the extent of peptic hydrolysis of beef protein at the average $C_H +$ of the ingested tissue (pH = 5.5); (4) the inhibitory effect of bile on peptic digestion.

The extent of hydrolysis was determined by fractional analyses of the digests, according to the method of Wasteneys and Borsook (2). In this method, protein is precipitated by a 2 per cent trichloracetic acid, proteoses by saturated sodium sulphate at 33° C., peptones by tannic acid, and polypeptides by alcohol. Alcohol precipitation was, however, carried out in only a few cases and in none of these was any measurable amount of amino-acid nitrogen found.

TABLE I
Rate of peptic hydrolysis of beef muscle protein
Suspension approximately 11 per cent

Experi-ment	Enzyme	Time	Hydroly-sis	Protein fractions			
				Protein N	Proteose N	Peptone N	Subpep-tone N
		seconds	*per cent*	*per cent of total N*	*per cent of total N*	*per cent of total N*	*per cent of total N*
A	1 per cent boiled pepsin	0		61	2	25	12
B	1 per cent active pepsin	30	14.9	52	13	14	21
C	1 per cent active pepsin	130	21.4	48	13	17	22
D	1 per cent active pepsin	180	26.8	45	18	14	23

PRELIMINARY IN VITRO EXPERIMENTS

1. *The rate of peptic hydrolysis of meat*

As a substrate for the experiment, 100 grams of lean beef were ground with sand in a mortar and 800 cc. of water added. This suspension was brought to a pH of 1.35 and to a temperature of 37.5° C. Sample A was removed, boiled pepsin [2] was added to a concentration of 1 per cent, and a fractional analysis made.

To the remainder, pepsin was added to a concentration of 1 per cent. Samples B, C and D were then removed 30, 130 and 180 seconds after the addition of pepsin. Immediately on removal the pepsin was destroyed by adding 1 cc. of concentrated sodium hydroxide.

The results of fractional analyses of these samples are given in Table I.

The concentration of active pepsin used in this experiment is relatively low. It is less than that which is often found in human gastric contents. Even in this low concentration pepsin acts *in vitro* with remarkable rapidity, 27 per cent of the protein having undergone hydrolysis in 180 seconds.

[2] Merck's commercial pepsin.

The rate of peptic hydrolysis is much more rapid with meat than with egg albumen, yet the relation between the different hydrolytic products, as hydrolysis proceeds, is similar.

2. *The acceleration of hydrolysis due to temperature when digests are heated to destroy the enzyme*

In view of the results obtained in the previous experiment, the possibility suggested itself that in the usual method for destroying enzyme action by rapid boiling, a transitory acceleration of hydrolysis might occur even though the time interval necessary to attain the temperature at which pepsin is destroyed be short.

In order to ascertain whether this acceleration is significant, a mixture of ground lean beef and water was brought to a pH of 1.2. This was divided into two portions. To one portion a solution of boiled pepsin in a concentration of 1 per cent was added. The mixture was boiled and submitted to fractional analysis. To the other sample, unboiled pepsin in the same concentration was added and the mixture was almost immediately boiled and analyzed. The results are given in Table II.

TABLE II

The degree of protein hydrolysis which occurs in a mixture of pepsin and macerated beef muscle during the time taken to raise the temperature of the mixture from room temperature to the boiling point

Fraction	Boiled pepsin and substrate raised to boiling point	Pepsin (active) added to substrate and raised to boiling point
	per cent	*per cent*
Protein N	56	16
Proteose N	10	36
Peptone N	27	27
Subpeptone N	7	21

Seventy-one per cent of the protein was hydrolysed. This experiment shows clearly that heating the digest in the presence of pepsin near the optimum pH for peptic hydrolysis causes quite marked hydrolysis, even though the time taken to raise the mixture from room temperature to that at which pepsin is destroyed, which is about 72° C. (3), is less than a minute. One must conclude, therefore, that under the conditions of this experiment boiling is an unsatisfactory method of inactivating the enzyme. It should be pointed out, however, that different conditions obtain when a sample is removed from the stomach an hour or more after the ingestion of 100 grams of lean beef and the material is boiled before making a fractional analysis. The enzyme and substrate have here been together for some time at a temperature of 37.5° C., and considerable hydrolysis has already taken place except in those cases where the pepsin content of the gastric juice is low. The speeding up of hydrolysis in the process of bringing the mixture to a boil would, in either condition, be minimized.

This is illustrated by the following experiment. The gastric contents of a patient were removed by stomach tube, one and one-half hours after ingestion of 100 grams of lean beef. The pH of the gastric contents was 1.65. They contained three times as much pepsin as was contained in the artificial digests. The gastric sample was divided into two parts. In one-half the pepsin was destroyed by boiling. In the other half it was destroyed by the addition of alkali. The fractional analyses are given in Table III.

TABLE III

A comparison of the nitrogen fractions in gastric contents after inactivation of the pepsin by boiling and by the addition of alkali

Fractions	Pepsin inactivated by boiling	Pepsin inactivated by alkali
	per cent	*per cent*
Protein N	23	25
Proteose N	41	40
Peptone N	20	22
Subpeptone N	16	13

If the sample in which the pepsin is inactivated by alkali be considered as the control, there is a negligible effect produced by meat. In most samples obtained from patients, even less than this amount of hydrolysis by heat would occur because in many cases the gastric contents contain less pepsin and the $C_H +$ is farther from the optimum for hydrolysis. One must conclude, therefore, that although boiling the gastric contents is not the best method for inactivating the pepsin, yet it may be used for gastric contents as the increased hydrolysis of the substrate during inactivation is negligible. In the majority of cases, there was only a limited supply of material obtained from the stomach, and as it was necessary to make several determinations on the same sample, the pepsin was inactivated by boiling.

3. *The peptic hydrolysis of beef protein at pH 5.5*

A question arises in the consideration of achlorhydria. Is there any digestion of meat at the pH of the gastric contents, usually 5.5 to 6, in this condition? In order to test this point a suspension of finely ground meat, adjusted to pH 5.5, was divided into two parts; to one was added boiled pepsin to a final concentration of 1 per cent. No hydrolysis was observed in 3¾ hours. To the other half, active pepsin was added in similar concentration. At the end of 3¾ hours 27 per cent of the protein had been digested. In spite of the possibility indicated by this experiment, it was found, as will be shown later, that very little digestion of protein actually takes place in the stomachs of achlorhydrics.

4. *The inhibitory effect of bile on peptic digestion*

Conflicting opinions are to be found in the literature as to the inhibitory action of bile on gastric digestion, and some experiments were carried out

in an endeavour to decide this question. The rate of peptic hydrolysis of 4 per cent egg albumen in the presence and absence of human bile from the gallbladder was determined, and in order to exaggerate any inhibitory effect of bile the amount of pepsin used in the experiment was relatively small while the amount of bile was considerably greater than that which is usually present in gastric contents as a result of regurgitation from the duodenum. The extent of hydrolysis during a period of four hours was measured. The inhibition of hydrolysis due to bile was, however, too slight to be significant.

HUMAN GASTRIC DIGESTION

In previous investigations of gastric digestion of beef muscle two general methods have been used: the formol titration of the gastric contents according to the method of Henriques and Sörenson (4), or some modification of that method; and the Van Slyke method of amino-nitrogen determination. The first method was used by Zunz (5), London (6), Christiansen (7) and Rehfuss (8). In general these observers found little apparent digestion in the stomach.

The relatively slight digestion found by these observers is probably accounted for by the fact that in peptic digests the ratio of free carboxyl or free amino-nitrogen to total nitrogen, even at equilibrium, is so small and the consequent error is so great, that little or no information can be gained as to the extent of changes which have occurred.

Apart from these investigations there have been few or no attempts at measurement of actual gastric digestion, and in studies of the relative digestibility of foods, workers have usually contented themselves with measurements of the emptying time of the stomach. In the present study of gastric digestion of beef protein the amount of digestion was followed by determining, at a given time after ingestion, the extent to which the protein had been hydrolyzed in the stomach into the various fractions.

It should be pointed out, however, that the amount of protein and nonprotein nitrogen in the gastric contents cannot be assumed to give an absolute measure of the extent to which beef muscle protein has been digested because of the unknown quantity of protein and nonprotein nitrogen contained in the saliva and other secretions of the gastro-intestinal tract, oral to the pylorus. They do, however, give the most accurate picture obtainable of actual gastric digestion of protein.

In this investigation, proven cases of pernicious anaemia and hospital patients suffering from other than gastro-intestinal diseases were used. In selecting the subjects, except in patients with pernicious anaemia, care was taken to avoid using any cases giving a definite history of previous gastro-intestinal disease or acute febrile states, debilitating disease, or any condition in which one might expect temporary achlorhydria. For the purpose of this study, achlorhydric individuals under observation were

divided into two groups: patients with achlorhydria with pernicious anaemia; and patients with achlorhydria without pernicious anaemia.

The subjects were prepared as follows: An ordinary meal was given at 4:45 p.m. on the preceding day, and on the day of the test no breakfast was given. At 9:00 a.m. the subject was given 100 grams of finely ground lean beef adequately flavoured with salt and pepper. The meat was fried to a light brown surface colour and 300 cc. of water was taken with the meat. Ingestion of the meat occupied from ten to fifteen minutes. The period of digestion was taken as the time from the beginning of ingestion to the time at which the removed gastric contents were heated to destroy the enzyme.

In order to obtain the stomach contents a large stomach tube was used. It was occasionally necessary to inject a small amount of water in order to clear the eye of the tube, and an attempt was always made to remove the entire content of the stomach at one time. This was immediately taken to the laboratory and a small amount put aside for pepsin and bile estimations. The remainder was boiled to destroy the pepsin. The boiled material was used for fractional analysis and also for the pH determination. It had already been shown that boiling did not appreciably affect the pH of the gastric contents.

Pepsin was estimated according to the method of Michaelis and Rothstein (9), by comparing the clearing of a suspension of a sulphosalicylic-acid-precipitated serum by a standard pepsin solution with the clearing effected by equal amounts of gastric contents in varying dilution. Human serum was obtained from the excess of serum remaining from samples of blood on which routine Wassermann tests were performed. It was necessary, in the estimations of pepsin, to use a filtrate of gastric contents because the turbidity produced by particles of meat interferes with the reading of the tubes. The dilutions were recorded as units of pepsin.

The standard pepsin solution was made from commercial scale pepsin which, when tested, complied with the U. S. Pharmacopoeia requirements. This solution contained a high percentage of glycerol. It was kept in the refrigerator and retained its activity for long periods of time.

The van den Bergh reaction was found to be sensitive for the detection of bile in the gastric contents. The unfiltered gastric contents were used for the test as it was found that less colour was produced in the filtrates than in the unfiltered material. This is believed to be due to adsorption of the bile on the particles of meat. After the addition of the diazo reagent and alcohol to the gastric contents the suspended particles were filtered off and the colour was estimated in the filtrate. The concentration of the sulphanilic acid and sodium nitrite was increased four times over that used in the standard van den Bergh test, as this was found to increase the sensitivity of the test. These bile estimations were only roughly quanti-

tative but one part of gallbladder bile in two thousand parts of a thick solution of finely ground beef gave a definitely positive test.

Assuming, according to clinical usage, that free hydrochloric acid is absent in cases where the pH is above 4 (that is, alkaline to dimethyl-amino-azobenzol (Toepfer's reagent)), then 90.5 per cent of the normal cases had free acid present in the gastric contents. Four per cent of the normals had gastric contents with pH values from 4 to 4.5, and an average titratable acidity of 17.2 cc. of N/10 acid per 100 cc. Sixty-five per cent of the cases had gastric contents with pH values ranging from 1.5 to 2.5, that is, in the range of pH for optimal peptic activity; for 11 per cent the pH values were within a range in which pepsin is relatively inactive. A few of these cases are not included in Table IV, which shows the correlation between the ex-

TABLE IV

The correlation between extent of hydrolysis of beef muscle protein and pH in human gastric contents

Range of pH	Number of cases	Per cent of total cases	Average pH	Average titratable acidity	Per cent cases showing bile	Average pepsin	Averages			
							Protein N	Proteose N	Peptone N	Subpeptone N
				cc. N/10		units	per cent of total N	per cent of total N	per cent of total N	per cent of total N
1–1.5	13	9	1.4	84.0	27	4.0	24	36	19	21
1.5–2	27	18	1.7	67.7	23	3.4	24	32	18	24
2–2.5	38	26	2.2	71.3	20	2.5	25	37	18	19
2.5–3	24	16	2.7	48.9	24	2.1	30	38	19	15
3–3.5	25	17	3.2	40.4	32	0.6	39	35	14	12
3.5–4	6	4	3.7	24.8	40	0.1	61	22	8	8
4–4.5	6	4	4.3	17.2	20	0.0	67	15	9	7
4.5–5	2	1	4.6	7.0	0	0.0	57	11	13	21
5–5.5	3	2	5.2	1.7	0	0.0	73	10	15	3
5.5–6	1	1	5.7		0	0.0	74	9	17	
6–6.5	0									
6.5–7	1	1	6.6		100	0.0	62	13	2	22
7–7.5	0									
7.5–8	1	1	7.7		100	0.0	71	17	6	6

tent of hydrolysis and pH in patients without history of gastric disease or pernicious anaemia. The 11 per cent of achlorhydric individuals found compares closely with the percentage observed by Bennett and Ryle (10). The titratable acidity varies with the true acidity from an average of 84 cc. of N/10 acid per 100 cc. of gastric contents at a pH of 1 to 1.7 cc. at a pH of 5. Methyl red was chosen as the indicator for the titration because its turning point is close to the normal pH of the meat fed to the patients. The large amount of acid secreted in response to the meat stimulus as compared with the stimulus of an Ewald test meal is noteworthy.

Of 129 " normal " subjects tested for bile, 25 per cent showed bile or

traces of bile in the gastric contents.   Of the 32 cases showing bile, 14 cases had only a trace.   Castle (11) states that bile appeared only occasionally in the gastric contents of normal students to whom meat was fed.   Judged by these results and those of Castle, duodenal regurgitation can neutralize gastric contents in only a small percentage of humans.   Boldyreff (12), working with dogs, states that the neutralization of gastric contents is normally effected by duodenal regurgitation, and Bolton and Goodhart (13) and Medes and Wright (14) also state that duodenal regurgitation is a normal means of neutralization of acid gastric contents in human subjects, but MacLean and Griffiths (15) seldom found CO_2 or trypsin in the gastric contents of humans, and Shay, Katz and Schloss (16) find that the neutralization of gastric contents by duodenal regurgitation, even when it occurs, is probably insignificant.

The amount of active pepsin in the gastric contents was found to diminish as the acidity decreased, and the highest pH at which active pepsin could be demonstrated was 3.5.   This is indicated in Table IV where the cases are grouped according to the pH of the gastric contents within a range comprising 0.5 of a pH.   The results are averages of the individual pH groups, and pernicious anaemia cases are excluded.

As would be expected, the amount of protein remaining undigested shows a marked increase at hydrogen ion concentrations less than a pH of 3.5, and the proteose, peptone and subpeptone correspondingly diminish as the acidity decreases.   It is not only the decreased acidity, however, which is responsible for the diminution in digestion, but also the absence of pepsin.   Of the 147 cases exclusive of pernicious anaemia, 86 per cent showed appreciable amounts of pepsin present, with a considerable digestion of protein.   Approximately one-fourth of the cases showed presence of bile.

There is considerable variation in all the factors in those cases in a given pH group.   This is illustrated in Table V, which includes cases comprising the first group (pH values 1 to 1.5) of Table IV.

In Table VI an attempt is made to compare the amount of digestion with its duration.   There is only a somewhat doubtful correlation shown between the length of time the beef muscle has been in the stomach and the actual digestion.   Obviously the factors of hydrogen ion concentration and pepsin content are of greater significance than duration of time in determining the amount of digestion effected.   When, however, the effect of time is examined at definite pH values, a less doubtful correlation is shown—Table VII.   These results are arranged in groups according to the pH of the gastric contents and within these groups the duration of digestion and amount of digestion are compared.   At a given pH there is a general tendency towards more digestion the longer the meat remains in the stomach.

TABLE V

Correlation between digestion and acidity in human gastric contents in the pH range 1–1.5

Case	Duration of digestion	pH	Titratable acidity	Bile	Pepsin	Protein N	Proteose N	Peptone N	Subpeptone N
	hours		cc. N/10		units	per cent of total N	per cent of total N	per cent of total N	per cent of total N
L	1.45'	1.23	100	++	8	28	26	24	22
I	2.0'	1.26				19	43	27	11
W	1.30'	1.30	87	0	1	23	43	19	15
R	2.15'	1.31	78	0	4	14	46	21	19
M	1.45'	1.34	102	0	2	23	46	13	18
K	1.50'	1.35	79	tr.	3	29	45	1	25
J	1.30'	1.37	94	0	3	30	31	20	19
W	2.20'	1.37	73	0	6	15	29	30	26
H	1.45'	1.38	84	tr.	6	28	48	5	19
F	1.55'	1.41	56	0	3	19	24	(57)	
B	1.55'	1.45	85	0	4	23	37	24	16
P	1.0'	1.46	52	0	4	(59)		12	29
C	2.0'	1.46	119			32	17	25	26

TABLE VI

Comparison of the amount of human gastric digestion with its duration

Protein digested	Average digestion	Average time	Longest time	Shortest time	Number of cases
per cent	per cent	minutes	minutes	minutes	
90–100	92	103	140	90	6
80–89	83	103	150	75	28
70–79	73	104	150	75	48
60–69	65	100	135	60	19
50–59	54	93	120	60	13
40–49	44	90	115	70	10
30–39	34	93	120	75	12
20–29	24	89	140	70	11
10–19	14	94	120	50	11
0–9	6	96	135	75	3

Correlation between the age of patients and amount of digestion, pH and titratable acidity was next studied. Table VIII gives the average digestion in the age groups.

In the second, third and fourth decades there is a gradual decrease in digestion, titratable acidity and free acidity as shown by increasing pH. In the fifth, sixth and seventh decades, however, there is more digestion and lower pH than in the fourth decade of life, whereas during the eighth no acidity, a high pH and low digestion prevails. The last two decades include, however, only an insignificant number of cases. Bloomfield and Keefer (17), in studying the secretion of the stomach, found no free acid in three out of five cases from 60 to 70 years of age. In grouping the

TABLE VII

Comparison of digestion in cases having same duration of digestion and pH

Number of cases	Time	Titratable acidity	Pepsin	Protein N	Proteose N	Peptone N	Subpeptone N
	hours	*cc. N/10*	*units*	*per cent of total N*	*per cent of total N*	*per cent of total N*	*per cent of total N*
			pH Group 1–1.5				
4	2.00–2.15	93.5	5	20	33	26	21
6	1.45–2.00	84.4	4	25	38	14	20
2	1.30–1.45	90.5	2	26	37	20	17
1	1.00–1.15	52.3	4	(59)		12	29
			pH Group 1.5–2				
2	2.15–2.30	50.2	5	9	16	(75)	
3	2.00–2.15	58.2	3	20	35	(45)	
7	1.45–2.00	77.0	4	28	34	17	22
9	1.30–1.45	68.3	4	31	29	30	19
3	1.15–1.30	64.0	3	23	39	15	23
			pH Group 2–2.5				
3	2.15–2.30	25.0	(2)	25	39	10	26
11	2.00–2.15	70.3	2	26	38	18	18
2	1.45–2.00			20	44	(36)	
			pH Group 2.5–3				
14	1.30–1.45	74.4	3	21	39	20	21
6	1.15–1.30	81.0	2	23	36	21	19
2	1.00–1.15	84.7	2	24	42	15	9
			pH Group 3–3.5				
2	2.15–2.30	28.6	trace	26	30	(44)	
5	2.00–2.15	40.6	1	34	48	4	16
2	1.45–2.00	18.3	trace	45	22	24	9
5	1.30–1.45	46.8	1	42	27	18	12
9	1.15–1.30	45.8	trace	39	27	12	11
1	1.00–1.15	15.9	trace	45	20	27	8
1	.45–1.00		1	31	60	(13)	

patients, they used the period of twenty years and found in their average a gradual decrease in the acidity of the gastric contents. If the above figures were calculated on the same basis, the average titratable acid between 20 and 40 years of age, and 40 and 60 years, would be about the same. Apparently in older subjects the acidity falls rapidly. Dedichen (18) working with over 100 subjects between the ages of 87 and 92 showed that

TABLE VIII

Correlation of age with gastric digestion, pH, and titratable acidity

Age group in years	Gastric contents			Number of cases
	Average pH	Average titratable acidity per 100 cc.	Average * protein digested	
		cc. N/10	per cent	
10–19	2.3	68	73	7
20–29	2.5	64	70	33
30–39	3.0	49	60	33
40–49	2.5	56	69	32
50–59	2.7	48	63	13
60–69	2.6	45	68	3
70–79	5.7	0	45	2

four-fifths of the men and three-fifths of the women had *achylia gastrica.*

Grouping the results on patients according to the clinical diagnosis on admission yielded very little information of value, but a few points of interest may be mentioned. Patients with disseminated sclerosis showed good digestion, normal acidity and normal amount of pepsin. Eleven osteoarthritic patients had, on the average, a normal activity of the stomach. This is contrary to the experience of Bell (19). He states that 37.5 per cent of the cases with osteoarthritis showed achylia gastrica; and 12.5 per cent showed low acid content. Two alcoholics showed less acidity and digestion than normal. Psychasthenics had normal secretion of acid and digestion of meat.

The extent of gastric digestion and the bile and pepsin in the gastric contents in achlorhydric individuals without pernicious anaemia[3] and in those suffering from pernicious anaemia is shown in Table IX. The average pH of the gastric contents was 6.7 with a range of from 5.75 to 7.53, and no titratable acid and no pepsin was found. The cases with pernicious anaemia showed less acidity than the achlorhydric cases and no combined acid, while several of the achlorhydrics showed small amounts

TABLE IX

Comparison of gastric activity in achlorhydrics with and without pernicious anaemia

Type	Number of cases	Average pH	Cases with bile	Pepsin	Protein N	Proteose N	Peptone N	Subpeptone N
					per cent of total N	per cent of total N	per cent of total N	per cent of total N
P.A.	23	6.7	4	0	75	7	6	11
Achlorhydric	13	4.9	2	0	67	12	10	8

[3] Subjects who have not suffered from acute illness recently so as to render them achlorhydric temporarily.

of combined acid.   The absence of free acid and the relative absence of titratable acid occurring in pernicious anaemia has been noted by many observers.   Bile was present in four out of twenty-three cases.   Medes and Wright (14) found that seven out of nine cases of pernicious anaemia showed bile in the gastric contents.

Very little actual hydrolysis of meat protein appears to take place in the stomach of cases with pernicious anaemia.   If the amount (approximately 10 to 14 per cent of sub-protein nitrogen) which is usually present in beef muscle be added to the average of 75 per cent of protein nitrogen, it appears that not much more than 10 per cent of the meat protein was hydrolyzed in the experimental period.

The achlorhydric cases without pernicious anaemia showed slightly more digestion.   Patients with pernicious anaemia and achlorhydrics without pernicious anaemia, as is well known, discharge ingested foods through the pylorus with much greater rapidity than other individuals.   The fact that achlorhydrics digest very little protein in the stomach suggests that measurements of " emptying time " may lead to entirely erroneous conclusions as to digestibility.

If there is anything in the theory that cases of pernicious anaemia are recruited from achlorhydric cases it is conceivable that there is some connection between the continued deprivation of peptic digestion and the disease.   A peculiarity common to the achlorhydric patients and those with pernicious anaemia is the relatively high proportion of the subproteose fraction in the gastric contents.   This suggests the possibility that such gastric digestion as does occur in these individuals is tryptic and not peptic in character, and is possibly in these subjects due to regurgitation of duodenal contents into the stomach.

In view of the great variation in the amount of digestion and in other factors shown by the different groups, an attempt was made to determine whether single individuals examined more than once under approximately the same conditions would show any constancy in behavior.   In Table X are given the results obtained with six individuals each examined on two or more occasions, one day or usually more apart.   While the hydrogen ion concentration and total acidity, and to a lesser extent the actual digestion, appear to be somewhat characteristic for the individuals, there is, nevertheless, considerable variation.   E. J. M., for example, who was examined on five separate occasions, always several days apart, had total acidities varying from 41 to 90 cc. N/10 per 100 cc.   And while the amount of active pepsin in the gastric contents was on four occasions relatively high, on one occasion, when the amount of digestion was correspondingly small, none could be detected.   This subject experienced no symptoms of gastric disturbance (other than that caused by withdrawal of the sample of gastric contents) or ill health during the period of the experiments.

TABLE X

Gastric digestion in single individuals on different days

Case	Duration of digestion	pH	Titratable acidity	Bile	Pepsin	Protein fractions			
						Protein N	Proteose N	Peptone N	Subpeptone N
	hours		*cc. N/10*		*units*	*per cent of total N*	*per cent of total N*	*per cent of total N*	*per cent of total N*
A	1.00'	3.0	63	0	2	15	44	33	8
	1.30'	3.6	47	0	tr.	52	27	18	2
	1.45'	2.4				20	44	14	22
B	1.15'	2.4	151	0	4	24	50	6	20
	1.45'	2.3	102	0	2	23	46	13	18
C	2.00'	1.7				29			
	2.00'	2.1				28	52	9	11
D	1.15'	3.4	36	0	tr.	59	27	3	11
	1.15'	2.4		0	1	20	21	40	19
E	1.20'	3.2		0	tr.	78	5	10	7
	1.15'	5.3		0	0	93	1	3	3
EJM	1.45'	2.5	74	++	3	30	29	27	14
	1.55'	2.7	41	+++	2	17	48	20	15
	1.55'	2.4	90	++	4	28	38	7	27
	1.15'	3.3	70	+++	0	67	19	7	7
	1.20'	2.7	51	+	2	24	45	14	17

The examinations of gastric contents made of a relatively large number of cases in the course of this study show, in general, a surprising uniformity in the extent of gastric digestion. It averages, one estimates, about 50 per cent. Even in cases of pernicious anaemia and in achlorhydrics, gastric contents which show no demonstrable pepsin and very low hydrogen ion concentration present from 10 to 20 per cent digestion. It seems that even from normal stomachs 40 to 50 per cent of ingested protein is on the average discharged through the pylorus in an undigested condition, but it is probable that the sojourn of the remaining 60 to 50 per cent in the bath of acid gastric juice may facilitate its digestion in the small intestine. Since under normal conditions at least 99 per cent of ingested protein is absorbed from the gastro-intestinal tract, the failure to complete peptic digestion in the stomach may have little significance so long as the digestive secretions of the small intestines are normal. In this connection, observations made on depancreatized dogs are of interest (20). In these dogs on the average only 60 per cent of ingested protein is absorbed from the gastro-intestinal tract, the remainder being eliminated unhydrolyzed in the faeces. If we may assume that with these animals, as with humans, roughly 50 per cent of the protein leaves the stomach unhydrolyzed, then the failure

to utilize more than 60 per cent of ingested protein in dogs may be due to the fact that in them only protein which has undergone peptic digestion is readily hydrolyzed to an absorbable form while ingested protein, which escapes this digestion, does not undergo further digestion in the intestine of the depancreatized animal. In such animals, therefore, gastric digestion is probably fundamental to proper nutrition.

SUMMARY AND CONCLUSIONS

1. The digestion of protein in the human stomach has been studied in a series of cases, and quantitative fractional analyses of gastric digests are reported.

2. Considerable peptic hydrolysis of meat can occur in the stomach in a relatively short time.

3. There is a very wide variation in the extent of hydrolysis of beef muscle protein in the normal individual. This variation occurs not only in different normal subjects, but also in the same individual.

4. Subjects with pernicious anaemia accomplish very little or no gastric digestion of meat.

5. No pepsin was demonstrated in the gastric contents of achlorhydric cases without pernicious anaemia nor of patients suffering from pernicious anaemia.

6. Achlorhydric cases without pernicious anaemia, however, showed a small amount of gastric digestion. A somewhat smaller amount was found in pernicious anaemia cases.

7. Under the conditions of these experiments, pepsin secretion and acid secretion appear to parallel each other in amount.

8. The pH of the gastric contents in individuals with apparently normal gastro-intestinal tracts, ranges from 1.23 to 6.63. No patients with pernicious anaemia had a pH below 5.75 for their gastric contents.

9. The titratable acidity ranged from 151 cc. of N/10 acid per 100 cc. of gastric contents to zero. As was true of the digestion of protein, it varied considerably in individual cases. In a few subjects on whom more than one determination was made variation was also found.

10. Bile was present in the gastric contents in measurable amounts in about 25 per cent of the normal subjects tested, and in 18 per cent of the subjects with pernicious anaemia.

11. It should be pointed out that these conclusions are based on observations of gastric digestion of meals consisting of meat only, and may be different for similar observations on a mixed dietary.

BIBLIOGRAPHY

1. Castle, W. B., Townsend, W. C., and Heath, C. W., Observations on the etiological relationship of achylia gastrica to pernicious anaemia. III. The nature of the reaction between normal human gastric juice and beef muscle leading to clinical improvement and increased blood formation similar to the effect of liver feeding. Am. J. M. Sc., 1930, 180, 305.

2. Wasteneys, H., and Borsook, H., A method for the fractional analysis of incomplete protein hydrolysates. J. Biol. Chem., 1924, 62, 1.

3. Borsook, H., and Wasteneys, H., The enzymatic synthesis of protein. II. The effect of temperature on the synthesizing action of pepsin. J. Biol. Chem., 1925, 62, 633.

4. Henriques, V., and Sörenson, S. P. L., Über die quantitative Bestimmung der Aminosäuren Polypeptide und der Hippursäure in Harne durch Formoltitration. Ztschr.. f. Physiol. Chem., 1910, 64, 120.

5. Zunz, E., Recherches sur l'azote titrable dans le contenu stomacal par la méthode de Sörensen au formol. Internat. Beitr. z. Path. u. Therap. d. Ernahrungstörungen, 1910, 2, 372.

6. London, E. S., Zum Chemismus der Verdauung im tierischen Körper. XXXVI. Über das Verhalten der Nucleoproteide im Magendarmkanal. Ztschr. f. Physiol. Chem., 1909, 62, 451.

7. Christiansen, J., Quoted by Rehfuss, M. E., p. 171 (8).

8. Rehfuss, M. E., The Diagnosis and Treatment of Diseases of the Stomach. W. B. Saunders and Co., Philadelphia, 1927, p. 171.

9. Michaelis, L., and Rothstein, M., Die Zerstörung von Lab und Pepsin durch Alkali. Biochem. Ztschr., 1920, 105, 60.

10. Bennett, T. I., and Ryle, J. A., Studies in gastric secretion. V. A study of normal gastric function based on the investigation of 100 healthy men by means of the fractional method of gastric analysis. Guy's Hosp. Rep., 1921, 71, 286.

11. Castle, W. B., Observations on the etiological relationship of achylia gastrica to pernicious anemia. I. The effect of the administration to patients with pernicious anaemia of the contents of the normal human stomach recovered after the ingestion of beef muscle. Am. J. M. Sc., 1929, 178, 748.

12. Boldyreff, W., The self regulation of the acidity of the gastric contents and the real acidity of the gastric juice. Quart. J. Exper. Physiol., 1914, 8, 1.

13. Bolton, C., and Goodhart, G. W., Duodenal regurgitation into the stomach during gastric digestion. Lancet, 1922, 202, 420.

14. Medes, G., and Wright, C. B., Studies on duodenal regurgitation. I. J. Clin. Invest., 1928, 6, 403.

15. MacLean, H., and Griffiths, W. J., The factors influencing the concentration of hydrochloric acid during gastric digestion. J. Physiol., 1928, 65, 63.

16. Shay, H., Katz, A. B., and Schloss, E. M., Experimental studies in gastric physiology. Evaluation of the rôle of duodenal regurgitation in the control of gastric acidity in man. Arch. Int. Med., 1932, 50, 605.

17. Bloomfield, A. L., and Keefer, C. S., Gastric acidity: relation to various factors such as age and physical fitness. J. Clin. Invest., 1928, 5, 285.

18. Dedichen, L., Anacidity in old persons. Acta med. Scandinav., 1924, Supplement 7, p. 345.

19. Bell, J. R., Notes on a consecutive series of 425 gastric analyses by the fractional method. Guy's Hosp. Rep., 1922, 72, 302.

20. Maltby, E. J., Utilization of ingested protein by depancreatized and normal dogs. Tr. Roy. Soc. Canada, 1931, 25, 201.